

Original Research Article

Molecular Detection and Typing of Genital Human Papillomavirus Among Female Medical and Nursing Students in Harare, Zimbabwe

ABSTRACT

Aims: to detect and type genital human papillomaviruses (HPVs) in young female medical and nursing students.

Study Design: Cross sectional study.

Place of Study: The study was conducted in Harare, Zimbabwe at the College of Health Sciences, University of Zimbabwe and Harare Central Hospital's School of Nursing in 2014.

Methodology: Self-collected cervico-vaginal swab specimens from female college students were processed and genomic DNA extracted. HPV-DNA was detected by consensus polymerase chain reaction (PCR) using two primer sets, MY09/MY11 and GP5+/GP6+. Positive PCR samples were typed by DNA sequencing and bioinformatics analyses.

Results: Cervico-vaginal swabs were self-collected from 125 students. The age range of the participants was 20-25 years with a mean age of first sexual activity of 19.22 years. HPV-related knowledge was low among the participants. One hundred and fourteen (114) out of the 125 swabs from the students had genomic DNA successfully extracted. Of these, 36 tested positive for HPV-DNA, giving a prevalence of 31.58%. Both high-risk (HPV 16, 18, 35, 45, 58, 53 and 56) and low-risk (HPV 6, 11, 40, 53, 54, 72, 81 and 86) genotypes were detected among the sexually-active students.

Conclusion: A high prevalence of HPV infection and lack of HPV-related knowledge in young medical and nursing students in Harare were observed. Further studies are necessary to establish the true prevalence HPV types in young women and the information generated will be useful in designing cervical cancer prevention policies in Zimbabwe.

Keywords: Genital human papillomavirus, students, infection, Zimbabwe.

1. INTRODUCTION

Genital human papillomavirus (HPV) is one of the most common sexually transmitted viruses worldwide [1]. It is the persistent infection with specific genital HPV genotypes that results in the development of genital and cervical cancers as well as warts [2]. Of the many HPV genotypes known to date, more than 40 of them infect the anogenital tract [3]. These genotypes that affect the anogenital mucosa are classified as either low- or high-risk genotypes depending on their ability to cause cancer and warts [4]. The high-risk HPV genotypes that may cause development of cervical cancer include HPV 16, 18, 31, 33, 35, 39, 45, 51, 56, 58, 59, 64, 68, 73 and 82 [5]. The majority of cervical cancer cases are however caused by HPV 16 and 18 [6]. The low-risk genotypes include HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and the majority of anogenital warts are caused by HPV 6 and 11 [7]. In Zimbabwe, cervical cancer is the most common malignancy in black women and accounts for 35.5% of all cancers [8]. Community-based studies on the genital human papillomavirus incidence and persistence in a cohort of HIV-negative women in Zimbabwe have shown HPV 16, 18, 31, 33 and 58 to be the most prevalent in Zimbabwean women [9]. In cervical cancer patients, the high-risk genotypes, HPV 16, 18, 31, 33, 52, 58 and 70 have been found to be prevalent in a number of studies in Zimbabwe [10]. Most studies on genital HPV in Zimbabwe have been done on adult women. Epidemiological data on the prevalence of genital HPV genotypes in adolescents and young healthy women in Zimbabwe are not available. The aim of the study was to detect and genotype genital HPVs in young female medical and nursing students at the University of Zimbabwe's College of Health Sciences and Harare Hospital's School of Nursing in Harare, Zimbabwe.

2. MATERIALS AND METHODS

2.1 Collection and processing of genital swabs from students

This was a cross-sectional study conducted on young and healthy sexually-active and non- sexually active female students based at the University of Zimbabwe's College of Health Sciences and the Harare Central Hospital's School of Nursing. The students aged between 17-25 years were recruited from the above-mentioned two institutions. A 10-minute recruitment Powerpoint presentation was given to the potential participants and those consenting were recruited into the study. Each student answered a questionnaire that provided demographic data and information such as sexual behaviour, age of first sexual encounter, number of sexual partners, as well as HPV-related knowledge. The self-collected genital/vaginal swabs were collected using sterile cotton swabs with transport media. Participants were instructed by the researcher on the correct procedure of self-collection of cervico-vaginal/genital swabs. The self-collection was done at their home. They were instructed to open the swab and insert the cotton tip about 4 cm into the vagina, turning it left and right on the vaginal wall to aid collection of vaginal excretions. They were then instructed to immediately place the swab in the tube containing the transport media avoiding putting it down or touching the surface of the skin. Participants were instructed to carry out self-collection procedures first thing in the morning and were issued with a self-sampling instructional sheet for reference when carrying out the procedure. Specimens were then collected by the researcher within 24 hours, and transported in biohazard labeled specimen bags at room temperature to the Department of Medical Microbiology laboratory. They were stored at 4 °C until extraction of DNA.

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54 **2.2 Genomic DNA extraction from the swabs**

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Genomic DNA from the swabs was extracted using the Quick-gDNA™ MicroPrep Kit (Zymo Research, USA) according to manufacturer's recommendations. Briefly, vaginal swabs were processed in batches of 20. The swabs were individually washed in 500 µl of lysis buffer in 1.5 ml Eppendorf tubes to dislodge cells from the cotton tip surface, followed by vortexing for 5 seconds. The vortexed mixtures were allowed to stand at room temperature for 10 minutes after which they were pipetted into Zymo-Spin IC Columns inserted into collection tubes. The columns were spun at 10 000 g for one minute and the collection tubes and flow through discarded. The columns were then placed in clean collection tubes followed by pipetting 200 µl of DNA pre-wash buffer into each column and spun at 10 000 g for one minute. To the spin columns, 500 µl of genomic DNA wash buffer was added and the columns spun at 10 000 g for one minute. DNA was eluted in a total elution buffer volume of 50 µl after incubation at 25-30 °C for 2 minutes, and high speed spinning for 30 seconds. The samples were then stored at -20 °C until PCR amplification.

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68 **2.3 HPV DNA amplification by consensus polymerase chain reaction**

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The HPV DNA amplification was performed by polymerase chain reaction (PCR) using consensus primers, MY09/MY011 set which amplified a 450 bp sequence within the L1 region of the HPV-DNA as well as the GP5+/GP6+ primer set that amplified a 150 bp sequence in the same region. The final reaction volume for each sample was 50 µl. A master mix for each PCR run was made while working on ice all the time. Each reaction contained 36.8 µl nuclease free water; 5 µl Taq buffer with Magnesium chloride ; 1 µl dNTPs (10mM); 1 µl Primer 1 (either MY09 or GP5+); 1 µl Primer 2 (either MY11 or GP6+); 0.2 µl Taq Polymerase (1U). To PCR tubes, 45 µl of the master mix was added and 5 µl of DNA template from each specimen to make the 50 µl reactions. The samples were run on the PTC-100™ Programmable Thermal Controller, MJ Research, Inc. The cyclor was programmed according to the primers being used. For the MY09/11 primer set, the program used was: initial denaturation 5 minutes at 95°, then 40 cycles with denaturation at 95°C for 30 seconds; annealing at 50°C for 30 seconds, extension for 45 seconds at 72°C, and a final 7 minute extension at 72°C. For the GP5+/6+ primer set: initial denaturation was 5 minutes at 95°, 40 cycles with denaturation at 95°C at 30 seconds; annealing at 44 °C for 30 seconds, extension for 45 seconds at 72°C, and a final 7 minute extension at 72°C. A house-keeping gene, the Trim5α (Trim5alpha) gene was also amplified for all samples. The final reaction volume was 25 µl with the pipetting scheme for the master mixes containing: 15.8 µl nuclease free water; 2.5 µl Taq buffer with MgCl2 ; 0.5 µl dNTPs (10mM); 0.5 µl Primer 1 (R136Q-F); 0.5 µl Primer 2 (R136Q-R); 0.2 µl Taq Polymerase (1U). Negative controls with master mix and nuclease free water in place of the template were used in every PCR run. The PCR program was an initial denaturation 5 minutes at 95°, 40 cycles of denaturation at 95°C for 30 seconds; annealing at 50°C for 30 seconds, extension for 45 seconds at 72°C, and a final 7 minute extension at 72°C. All PCR products were checked by 2.0% agarose gel electrophoresis.

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93 **2.4 DNA sequencing, HPV genotype identification and data analysis**

DNA sequencing of the HPV DNA-positive PCR products was performed at Inqaba Biotech (Pretoria, South Africa) by the Sanger sequencing method on an automated ABI-prism 3100 Genetic Analyser (Applied Biosystems, California, USA). DNA sequences were analyzed using the Geneious Basic program (Biomatters, USA). Nucleotide sequence searches in public genbanks were done using the NCBI online BLAST tool program (<http://www.ncbi.gov/BLAST>). Nucleotide sequences were assigned HPV genotypes if the sequence corresponded 100% with a known HPV genotype available in the genbanks. Published literature was used to separate the genotypes into low- and high- risk genotypes upon data analysis. The data was captured, sorted and cleaned in Microsoft Excel 2011. Data were exported to Stata 11 software for both descriptive analysis of the demographic data and bivariate analysis on the diagnostic performance the PCR primers in detecting HPV infection, prevalence and genotyping.

3. RESULTS

3.1 Demographic data of student participants

The study population consisted of a total of 125 young female students who were recruited into the study. Of the 125, 78 (62%) were from the University of Zimbabwe's College of Health Sciences and were mainly MBChB Part III students. The other 47 (38%) were nursing students from the Harare Central Hospital's School of Nursing. All the participants reported being of African descent and were black. The mean age of the participants was 22.4 years. The age range of participants was from 20 to 25 years. Of the 125 women, 80 (64%) reported to be sexually-active with an average age of their first sexual activity at 19.22 years (95% CI: 18.73 – 19.72 years), while 45 (36%) reported not having had sex before. The socio-demographic data of the participants are summarized in Table 1. The sample drawn from Harare Central Hospital School of Nursing and UZ College of Health Sciences revealed that 39% of the 125 young women had more than one sexual partner. The majorities were single women (57%) and sexually active (64%). Only a few of the young women reported a positive STI infection history (8.8%) and there was an HPV knowledge deficiency. An HPV PAP testing unmet need was detected with 27% having reported having one done while 89% had never had one.

Table 1. Socio-demographic characteristics of the study population (n=125)

Outcome	Proportion n (%)	95% Confidence Interval	P value (^a versus ^b)
No. of Sexual partners			
Zero or one sexual partner	35 (61.5 ^a)	51.4 – 71.7	< 0.0001*
Many sexual partners	56 (38.5 ^b)	28.3 – 48.6	
Marital Status			
Engaged	2 (2.2 ^a)	0.9 – 5.4	< 0.0001*
Married	31 (33.7 ^b)	23.7 – 43.7	
Other	2 (2.2 ^a)	0.9 – 5.4	
Single	86 (57.3 ^b)	46.8 – 67.8	
Separated	4 (4.5 ^a)	0.1 – 8.9	
Sexual Activity			
Yes	80 (64.0 ^a)	55.5 – 72.5	< 0.0001*
No	45 (36.0 ^b)	27.5 – 44.5	
STI History			
Yes	8 (8.8 ^a)	2.9 – 14.7	< 0.0001*
No	83(98.2 ^b)	85.3 – 97.1	
Non-response	34		
HPV Knowledge			
Lacking knowledge	2 (1.6 ^b)	0.6 – 3.9	< 0.0001*
Knowledgeable	91 (73.4 ^a)	65.5 – 81.3	
Thinks men are not infected	17 (13.7 ^b)	7.6 – 19.8	
Thinks there is cure	14 (11.3 ^b)	5.6 – 16.9	

Previous PAP Exam

Yes	14 (11.3 ^a)	5.6 – 16.9	
No	110 (88.7 ^b)	83.1 – 94.4	< 0.0001*
Non-response	1		

* statistically significant at 95% confidence level

^a versus ^b pairwise comparison for significant difference

3.2 HPV DNA amplification from swabs

Total genomic DNA was successfully isolated from all the 125 swab samples from the students. To check the quality of DNA preparations, a house-keeping gene, TRIM5α gene was amplified (Fig. 1). The majority of the samples showed amplification of the gene. Gel electrophoresis showed the presence of the expected PCR band of 526 bp (Fig 1). There was no amplification in only 11 (9%) of the 125 DNA preparations. These were excluded from further for the presence of HPV DNA.

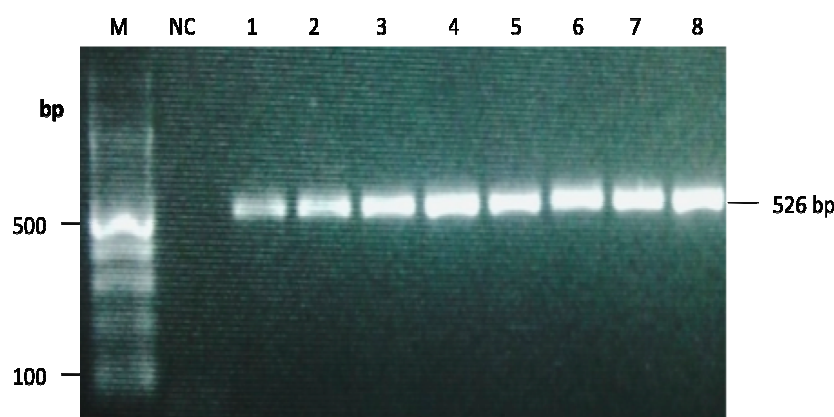


Fig. 1. A representative gel photograph showing amplification of a house-keeping gene, TRIM5α gene from swabs. Lane M: DNA marker, NC: polymerase chain reaction negative contro, Lanes 1-8: successfully amplified Trim5α gene

The 114 valid samples which tested positive for the Trim5α gene were further tested for the presence of HPV DNA using MY09/11 and GP5+/GP6+ primer sets. Samples that were positive for HPV DNA using the MY09/11 primers showed the presence of an expected 450 bp band on gel electrophoresis (Fig. 2). Samples that were positive for HPV DNA using the GP5+/GP6+ primers showed the presence of an expected 150 bp band on gel electrophoresis (Fig. 3). A summary of the results of HPV DNA amplifications are in Table 2. Briefly, the MY09/11 primer set gave an HPV prevalence of 36/114 (31.58%) while GP5+/6+ primer set gave a prevalence of 26/114 (22.8%). Overall, a total of 36/114 samples (31.58%) tested positive for HPV-DNA with both MY09/11 and GP5+/6+ primer sets.

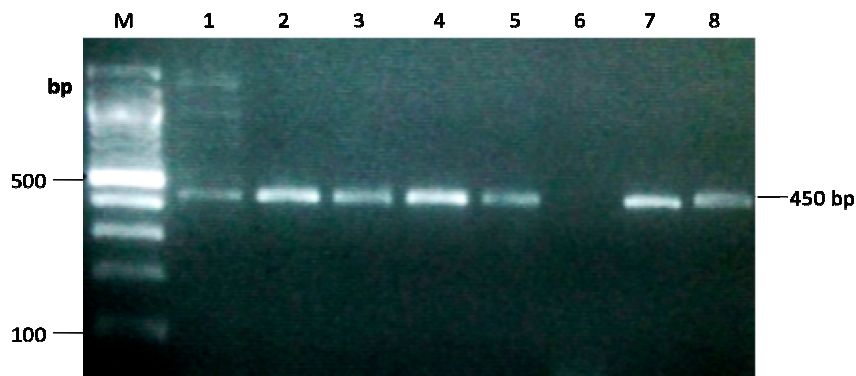


Fig. 2. A representative gel showing amplification of HPV DNA from swabs using MY09/11 primer sets. Lane M: DNA marker, Lanes 1-5, 7 and 8: amplified HPV DNA, Lane 6: no amplification of HPV DNA.

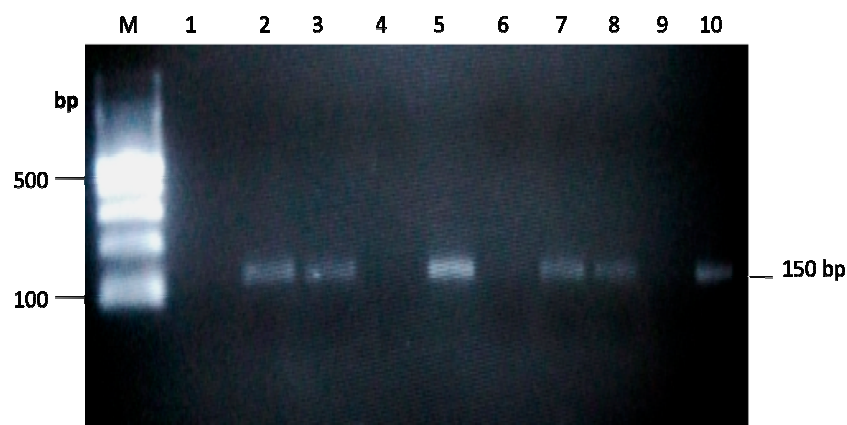


Fig. 3. A representative gel showing amplification of HPV DNA from swabs using GP5+/GP6+ primer sets. Lane M: DNA marker, Lanes 2,3,5,6,7 and 10: amplified HPV DNA, Lanes 1,4,6 and 9: no amplification of HPV DNA.

Table 2. Detection of genital HPV by two PCR primer sets (MY09/11 and GP5/6) in students (n=114)

Outcome	Frequency (%)
MY09/11 primer set	
Positive	36 (31.58)
Negative	78 (68.42)
Total	114 (100)
GP5+/6+ primer set	
Positive	26 (20.81)
Negative	88 (77.19)
Total	114 (100)
Overall HPV status	
Positive	36 (31.58)
Negative	78 (68.42)
Total	114 (100)

3.3 Genital HPV genotypes detected in medical and nursing students

The 36 PCR samples that were positive for HPV DNA by either MY09/11 or GP5+/6+ primer sets were sequenced. Out of these samples, 29 gave sequence data that could be used for identification of HPV genotypes. Analysis of the sequence data showed that 10 out of 29 (37.93%) had infection with a high-risk HPV genotypes; 1 out of 29 (3.45%) with a probable high risk HPV genotypes; 8 out of 29 (27.5%) were infected by a low-risk HPV genotypes; 2 out of 29 (6.90%) were infected by HPVs of unknown or not yet well-characterized genotypes. Mixed infections with either a high-risk or low-risk genotype were found in 13 samples. The 17 known HPV genotypes detected in the students were HPV 81, 53, 6, 90, 72, 40, 11, 86, 54, 16, 68, 45, 18, 58, 35, 56 and 67. Three samples had HPV sequences that could not align 100% with any genotype sequences in the Genbanks. Of the total (17 known and 3 unknown) 7 out of 20 (35%) were high-risk (HPV genotypes 16, 18, 35, 45, 58, 53, 56), 8 out of 20 (40%) were low risk (HPV genotypes 6, 11, 40, 53, 54, 72, 81, 86) 1 out 20 (5%) was

probable high-risk (HPV genotype 67), 1 out of 20 (5%) was probable low-risk (HPV genotype 90), and 3 out of 20 (15%) were of unknown HPV genotypes. Probable high- or low-risk genotypes are those that have not been classified into either category but assumed to be either while more research is being carried out around them.

4. DISCUSSION

Genital HPV infections are very common in sexually active individuals [11]. Most epidemiological studies on the prevalence of these infections have only been done in old women especially those with cervical cancer. Data on genital HPVs in adolescents and young women are still very scarce. In this study, we investigated the prevalence of genital HPV genotypes in young female medical and nursing students in Harare, Zimbabwe. Analysis of data from the administered questionnaire showed that more than half of the participants reported being sexually active with 38.5% of the subjects having had more than one sexual partner. This aspect was investigated in our study subjects because exposure to multiple partners is a well-known risk factor for genital HPV infection [12]. Overall, sexual activity is high in the age-group and hence the risk of HPV infection is also very high.

The socio-demographic and HPV-related knowledge analysis of the participants showed a statistically significant association between HPV infection and HPV knowledge as well as early age of first sexual encounter. After looking at the HPV related knowledge responses, 13.7% of participants believed that men could not be infected with HPV thus implying they did not understand it as being a sexually transmitted infection. This meant that the women were more likely to indulge in unprotected sex with their partners as well as not being able to link the presence of cervical lesions at screening to being associated with a viral infection from their sexual partners. Such lack of knowledge put the women at a higher risk of getting infected with multiple HPV genotypes if their partner had multiple sex partners. Some of the participants (11.3%) answered that there was a cure for HPV showing a misunderstanding between the infection being vaccine-preventable and the symptoms manageable as compared to being curable. Lack of HPV-related knowledge in young university or college female students has also been reported elsewhere in Africa. A study of 368 female students aged 16-29 years at the University of Lagos in Nigeria showed a very low knowledge of HPV infection and HPV-related diseases [13]. Only about 18% and 14% of the students said they had ever heard of HPV infection and HPV vaccines respectively [13]. A study in South Africa showed that the knowledge of female university students about cervical cancer and HPV was insufficient, although the students had positive attitudes toward getting vaccinated [14]. In another study of 86 women aged 18-44 years in a township in Johannesburg, South Africa also showed that they had limited HPV-related knowledge [15]. A study at a tertiary hospital in South Africa also showed that nurses had poor knowledge of HPV infection and vaccine [16]. At a regional hospital in Tanzania, less than half of the nurses had enough knowledge of HPV and cervical cancer [17]. This lack of knowledge in our targeted group mainly comprising of medical and nursing students was unexpected as one would believe they were taught during their tertiary courses. There was also a low level of HPV screening with 88.7% of the participants never having had a Pap smear examination. A continued lack of knowledge would result in an even higher prevalence of HPV in young women in Zimbabwe and consequently increased morbidity and mortality due to HPV related cervical cancer.

Polymerase chain reaction (PCR) is a molecular method which is increasingly being used to detect HPV. In this study, we first checked the quality of genital samples collected from students by amplifying a house-keeping human gene. The fact that the house-keeping gene amplified in most samples (Figure 1) showed that there were sufficient cells self-collected by the students for HPV DNA analysis. PCR of the L1 region of the HPVs was targeted using the consensus HPV-specific primers (MY09/11 and GP5+/6+ sets). The MY09/11 primer set detected more HPV DNA than the GP5+/6+ primers set. In this study, a greater number of positive samples were observed using MY09/11 primers when compared to GP5+/6+ primers. From 114 samples, 36 (31.58%) were positive for HPV-DNA. MY09/11 primers gave a prevalence of 36/114 (31.58) while GP5+/6+ gave a prevalence of 26/114 (22.8%). However there was no statistical difference between the two primer sets used for the consensus PCR ($p < 0.001$). The level of agreement between MY09/11 PCR and GP5+/6+ primers had an un-weighted kappa value of 80.62 % which showed a very good strength of agreement. The MY09/11 primers are degenerate and can therefore pick more HPV genotypes than the GP5+/GP6+ primers which are not degenerate. The current study showed an overall HPV-DNA prevalence of 31.58% which was high. A study in a group of young women (18 -35 years) in New York, USA, gave a prevalence of 28.9% [18]. Another study in Brazil showed a prevalence of genital HPV to be 25.3 %

(67/265) in university students, and multiple infections were common [19]. In this study, both high-risk and low-risk HPV genotypes were detected in sexually-active medical and nursing students. Most of the HPV genotypes found in the subjects were also found in a cohort of HIV-negative Zimbabwean women [9]. Both low- and high-risk genotypes found in healthy Zimbabwean women and students would predispose them to the development of genital warts and cervical cancer. Most of HPV genotypes found in this study were the same as those found in young women in other studies elsewhere. In the Brazilian study of female university students, the most prevalent genotypes were HPV 61, 82, 16, 59, and 6 [19]. A study in young women (mean age, 21 years) in Kwazulu-Natal, South Africa showed that the predominant high-risk genital genotypes were HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56 and 58 [20].

This study has some implications for Zimbabwe. Currently with effect from September 2014 the Ministry of Health and Child Care of Zimbabwe launched a pilot project under the Expanded Programme of Immunisation where young school girls from two provinces in Zimbabwe received the HPV vaccine doses. The lack of epidemiological and prevalence data in the young women in Zimbabwe has made unclear the target population for vaccination for full implementation of the programme. Studies such as this one in the 17-25 year old college and university students has shown high sexual activity as well as high HPV prevalence. These results may indicate a cause of concern in the effectiveness of the vaccination programme especially since they may already be highly infected. According to some literature, young women aged 25 years or less would be eligible for vaccination and in this study, HPV prevalence was already high prior to vaccination. This study on the students in Harare is the first study done in Zimbabwe on young females and more studies still need to be done to help add to the knowledge on the prevalent genotypes.

5. CONCLUSION

Prevalence of high-risk human papillomavirus (HPV) was found to be high in young sexually active university and college students in Harare. In these students, lack of HPV-related knowledge was observed. An increase in access to screening and education about HPV and preventing cervical cancer is needed. Further epidemiological studies on HPV genoprevalence, with increased sample sizes are required in Zimbabwe. Data generated will be useful in designing HPV and cervical cancer prevention policies.

ETHICAL APPROVALS

The study was approved by the Joint Research Ethics committee of Parirenyatwa Hospital and the University of Zimbabwe College Of Health Sciences (JREC/41/14) and the Medical Research Council of Zimbabwe (MRCZ/B/649). The experiments were performed in accordance with the ethical standards laid down by the committee and council. All students signed informed consent forms.

REFERENCES

1. Monk BJ, Tewari KS. The spectrum and clinical sequelae of human papillomavirus infection. *Gynecol Oncol*. 2007; 107(2 Suppl 1):S6-13.
2. Rapose A. Human papillomavirus and genital cancer. *Indian J Dermatol Venereol Leprol*. 2009; 75(3):236-43.
3. Sanjosé S, Bosch FX, Muñoz N. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*. 2003; 348(6):518-27.
4. Brendle SA, Bywaters SM, Christensen ND. Pathogenesis of infection by human papillomavirus. *Curr Probl Dermatol*. 2014; 45:47-57.
5. Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ; International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*. 2003; 348(6):518-27.

- 281 6. Bosch FX, Manos MM, Muñoz N, Sherman M, Jansen AM, Peto J, Schiffman MH, Moreno V,
282 Kurman R, Shah KV. Prevalence of human papillomavirus in cervical cancer: a worldwide
283 perspective. International biological study on cervical cancer (IBSCC) Study Group. *J Natl Cancer*
284 *Inst.* 1995; 87(11):796-802.
- 285 7. Steben M, Garland SM. Genital warts. *Best Pract Res Clin Obstet Gynaecol.* 2014; 28(7):1063-
286 73.
- 287 8. Chokunonga E, Borok MZ, Chirenje ZM, Makunike-Mutasa R, Ndlovu N, Nyakabau AM, Vuma S.
288 Pattern of cancer in Zimbabwe. Zimbabwe National Cancer Registry. 2014 Annual Report.
289 Harare, Zimbabwe; 2016.
- 290 9. Fukuchi E, Sawaya GF, Chirenje M, Magure T, Tuveson J, Ma Y, Shiboski S, Da Costa M,
291 Palefsky J, Moscicki AB, Makunike-Mutasa R, Chipato T, Smith-McCune KK. Cervical human
292 papillomavirus incidence and persistence in a cohort of HIV-negative women in Zimbabwe. *Sex*
293 *Transm Dis.* 2009; 36(5):305-11.
- 294 10. Chin'ombe N, Sebata NL, Ruhanya V, Matarira HT. Human Papillomavirus genotypes in cervical
295 cancer and vaccination challenges in Zimbabwe. *Infect Agent Cancer.* 2014; 9:16.
- 296 11. Ault KA. Epidemiology and natural history of human papillomavirus infections in the female genital
297 tract. *Infect Dis Obstet Gynecol.* 2006; 2006 Suppl:40470.
- 298 12. Chelimo C, Wouldes TA, Cameron LD, Elwood JM. Risk factors for and prevention of human
299 papillomaviruses (HPV), genital warts and cervical cancer. *J Infect.* 2013; 66(3):207-17.
- 300 13. Makwe CC, Anorlu RI, Odeyemi KA. Human papillomavirus (HPV) infection and vaccines:
301 knowledge, attitude and perception among female students at the University of Lagos, Lagos,
302 Nigeria. *J Epidemiol Glob Health.* 2012; 2(4):199-206.
- 303 14. Hoque ME, Ghuman S, Hal GV. Human Papillomavirus vaccination acceptability among female
304 university students in South Africa. *Asian Pac J Cancer Prev.* 2013; 14(8):4865-9.
- 305 15. Francis SA, Nelson J, Liverpool J, Soogun S, Mofammere N, Thorpe RJ Jr. Examining attitudes
306 and knowledge about HPV and cervical cancer risk among female clinic attendees in
307 Johannesburg, South Africa. *Vaccine.* 2010; 28(50):8026-32.
- 308 16. Hoque ME, Monokoane S, Van Hal G. Knowledge of and attitude towards human papillomavirus
309 infection and vaccines among nurses at a tertiary hospital in South Africa. *J Obstet Gynaecol.*
310 2014; 34(2):182-6.
- 311 17. Urasa M, Darj E. Knowledge of cervical cancer and screening practices of nurses at a regional
312 hospital in Tanzania. *Afr Health Sci.* 2011; 11(1):48-57.
- 313 18. De Almeida FG, Machado AP, Fernandes CE, Ferreira AT, Padovani CT, Tozetti IA. Molecular
314 epidemiology of the human papillomavirus infection in self-collected samples from young women.
315 *J Med Virol.* 2014; 86(2):266-71.
- 316 19. Vieira RC, Monteiro Jdo S, Manso EP, Dos Santos MR, Tsutsumi MY, Ishikawa EA, Ferrari SF,
317 Lima KV, de Sousa MS. Prevalence of type-specific HPV among female university students from
318 northern Brazil. *Infect Agent Cancer.* 2015; 10:21.
- 319 20. Ebrahim S, Mndende XK, Kharsany AB, Mbulawa ZZ, Naranbhai V, Frohlich J, Werner L,
320 Samsunder N, Karim QA, Williamson AL. High Burden of Human Papillomavirus (HPV) Infection
321 among Young Women in KwaZulu-Natal, South Africa. *PLoS One.* 2016; 11(1):e0146603.